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To estimate the concentration of protein using Biuret method. The is a colorimetric estimation technique that is specific for proteins and peptides used for estimation of protein. The coppers salt reacts with the two more peptide bonds under alkaline condition result in purple complex. The intensity of the formed color complex is proportional to the number of peptide bonds or the concentration of the protein sample which is read at (540–560 nm). using spectrophotometry against blank solution. Fig: Estimation of protein by Biuret method Biuret Reagent: Dissolve 1.5 gm of CuSO4 is dissolved along with 4.5 gms of Na-K tartrate (Rochelle salt) in 250 mL 0.2 NaOH solution. Finally, add 2.5 gms of Potassium Iodine (KI) and make up the volume to 500 mL with 0.2 N NaOH. The Rochelle salt acts as a chelating agent and stabilizes the copper (II) ions). Protein Standard: 1 mg BSA/ml. Test tubes, Pipettes, Colorimeter, etc., Prepare aliquots of 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard BSA solution in to the series of labeled different clean, sterile, and dry test tubes. 1 ml of the given test sample in placed in another test tube and labeled as “Test”. Likewise, 1 ml of distilled water is another tube and labelled as “Blank”. Add distilled water to all the tubes and make up the volume to 1 ml except in test solution and blank. Now, add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled ‘Blank’ and ‘Test”’. Mix the contents of the tubes by vortexing / shaking the tubes and warm at 37 ºC for 10-15 minutes and cool The developed color after the reaction will be record the absorbance at 540 nm against blank. Finally, a standard curve will be potted by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis. Then from this standard curve calculate the concentration of protein in the given test sample. The concentration of the given test solution can also be calculated using the given formula: Albumin-based or albumin-linked calibrators cause a positive bias in serum proteins assayed by the biuret method. Chromý V, Sváchová L, Novosád L, Jarkovský J, Sedlák P, Horák P, Dobrovolná H, Hlavácová B. Chromý V, et al. Clin Chem Lab Med. 2009;47(1):91-101. doi: 10.1515/CCLM.2009.011. Clin Chem Lab Med. 2009. PMID: 19117409